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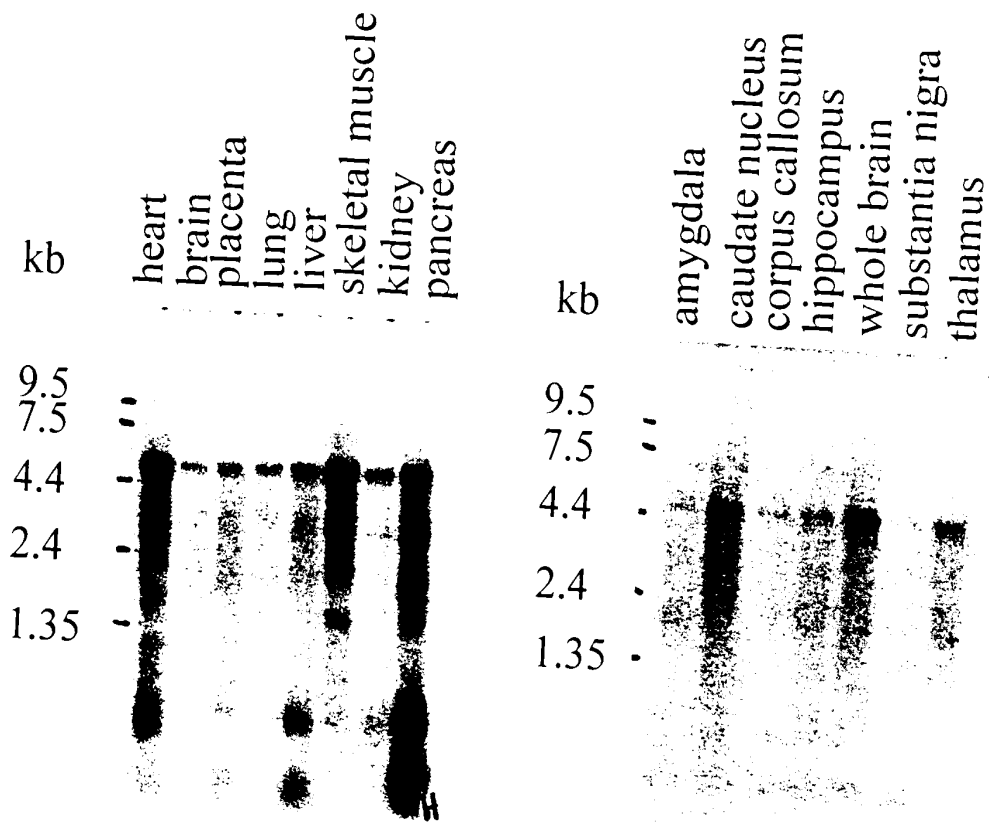
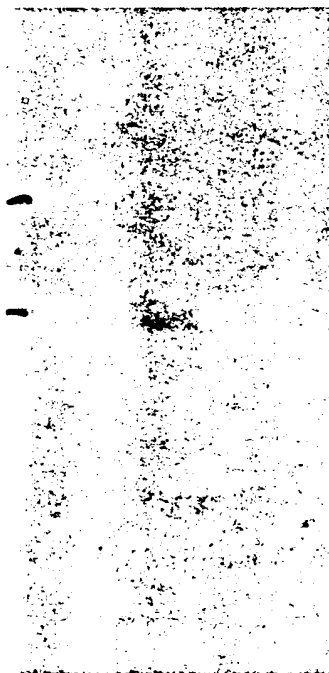


Fig. 1

28S

18S



HiB5

HiB5pUbi1zNBN22

HiB5pUbi1zGDNF14

Fig. 2

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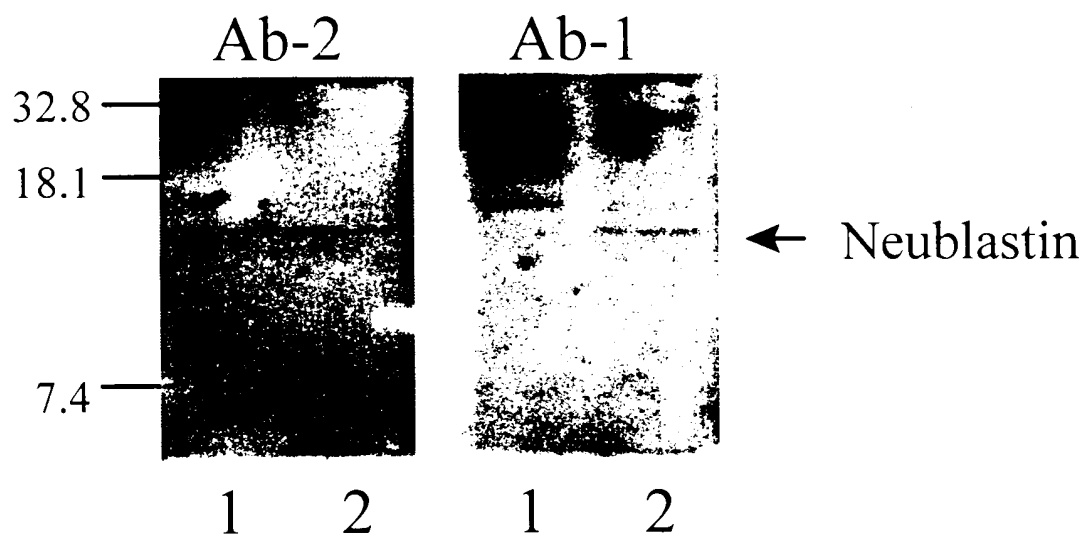
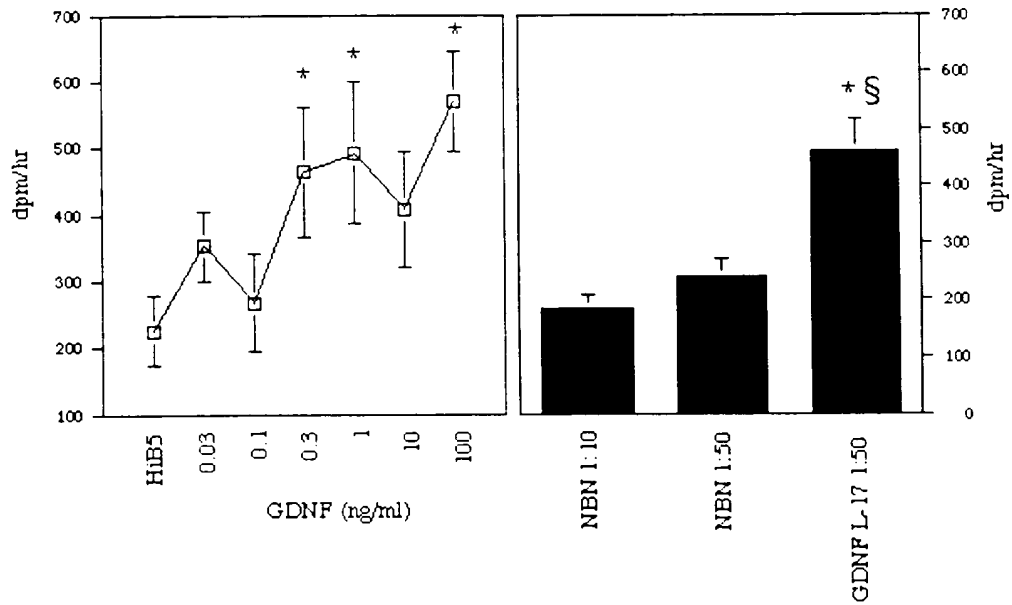
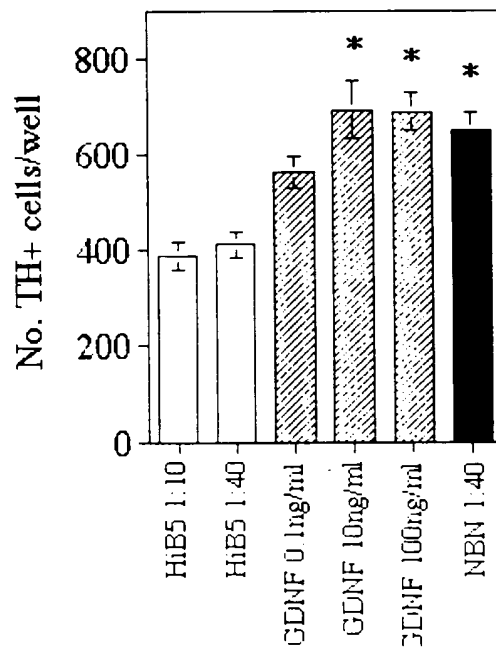


Fig. 3

NBN1 ChAT bioassay



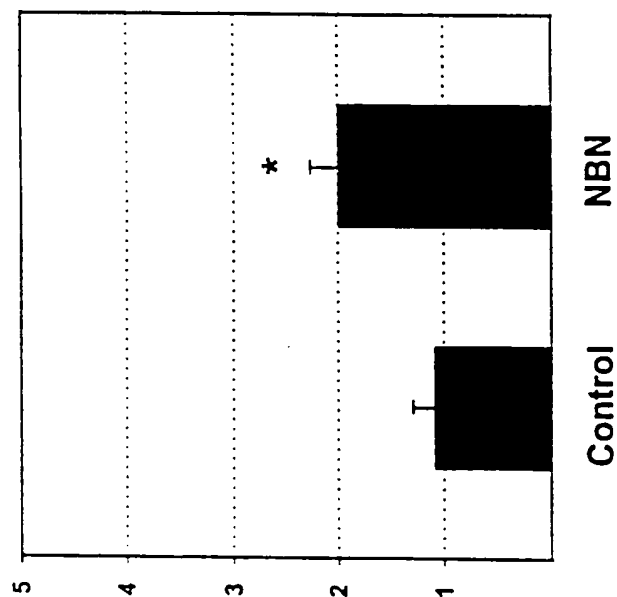
TH+ cell number at DIV 7



Figs. 4A, 4B and 4C

5118

Dopamine (pmol/ml) - day 12



Dopamine (pmol/ml) - day 21

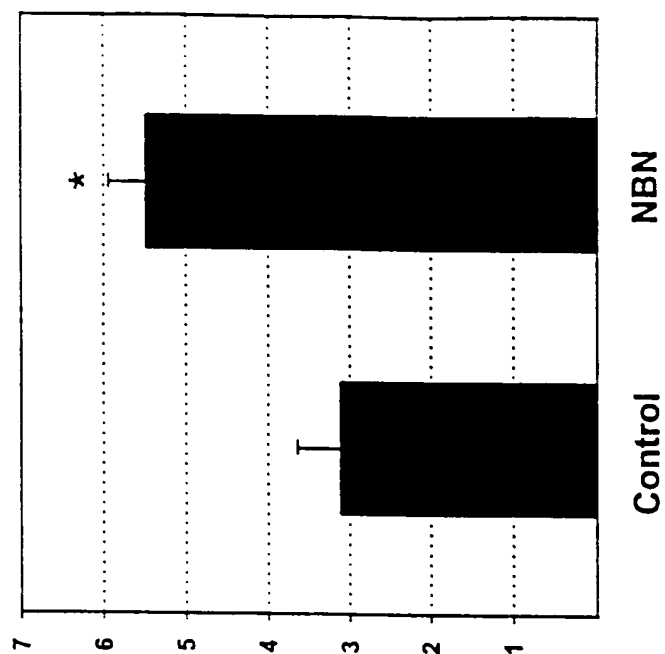


Fig. 5A and 5B

6118

TH-ir cells per culture

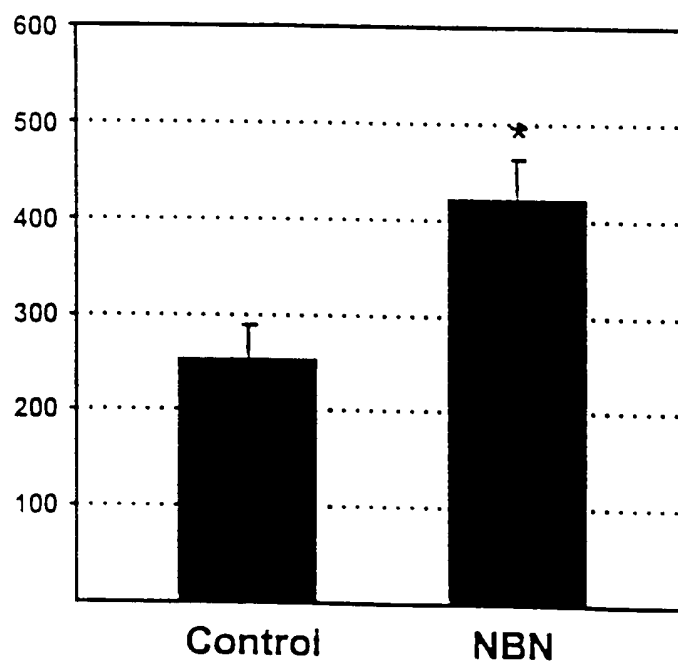


Fig. 5C

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%FG lesion/intact

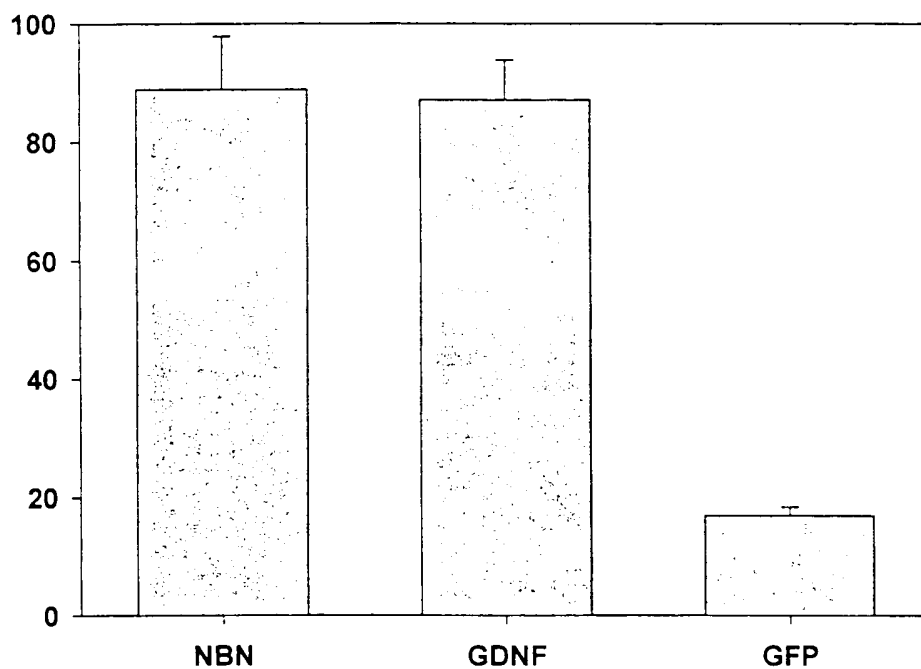
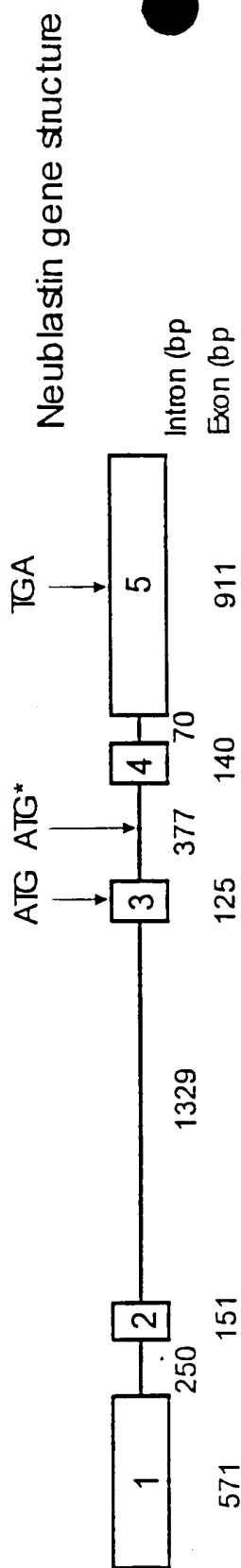
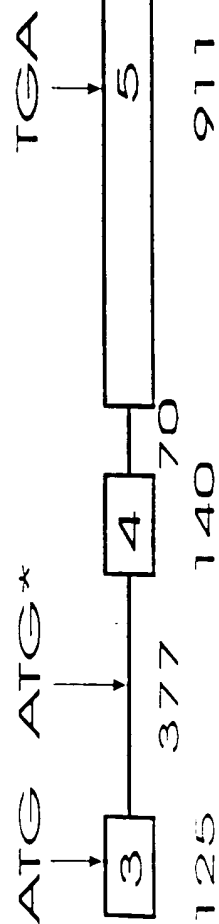


Fig. 6

8114



Seq. Id number 8



290 bp fragment identified in TBLASTN search

Genomic neublastin sequence amplified

NBN primers

Seq. Id number 17
Seq. Id number 18
Seq. Id number 21
Seq. Id number 22
Seq. Id number 23
Seq. Id number 24
Seq. Id number 25
Seq. Id number 26

Fig. 7

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Alignment of Neublastin primers used in Rapid-Screen with
homologous regions in other GDNF ligands

| | |
|---------------------------------|--------------|
| 5' -C CTG GCC AGC CTA CTG GG-3' | SEQ ID No 17 |
| G CTG GCC CGG CTG CAG GG | persephin |
| G CTG CGA CGA CTG CGC CA | neurturin |
| A TTG AAA AAC TTA TCC AG | GDNF |

| | | |
|--------------------|----------------|--------------|
| 5' -AA GGA GAC CGC | TTC GTA GCG-3' | SEQ ID No 18 |
| TA GGC CAC GTC | GGT GTA GCG | persephin |
| AA GGA CAC CTC GTC | CTC GTA GGC | neurturin |
| AA CGA CAG GTC ATC | ATC AAA GGC | GDNF |

conserved nucleotides shown in **bold**

Fig. 8

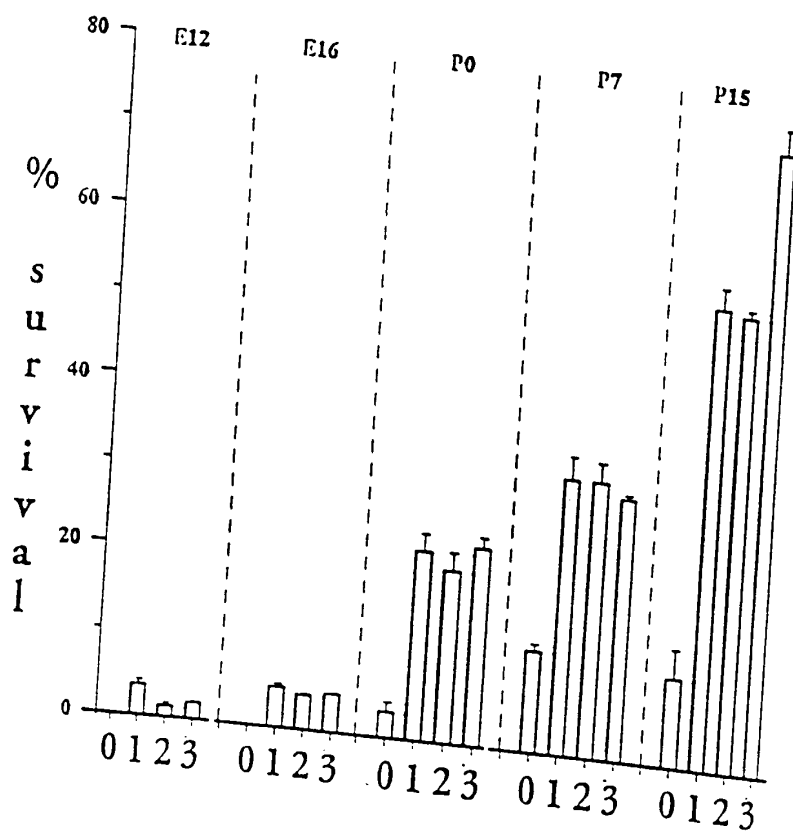
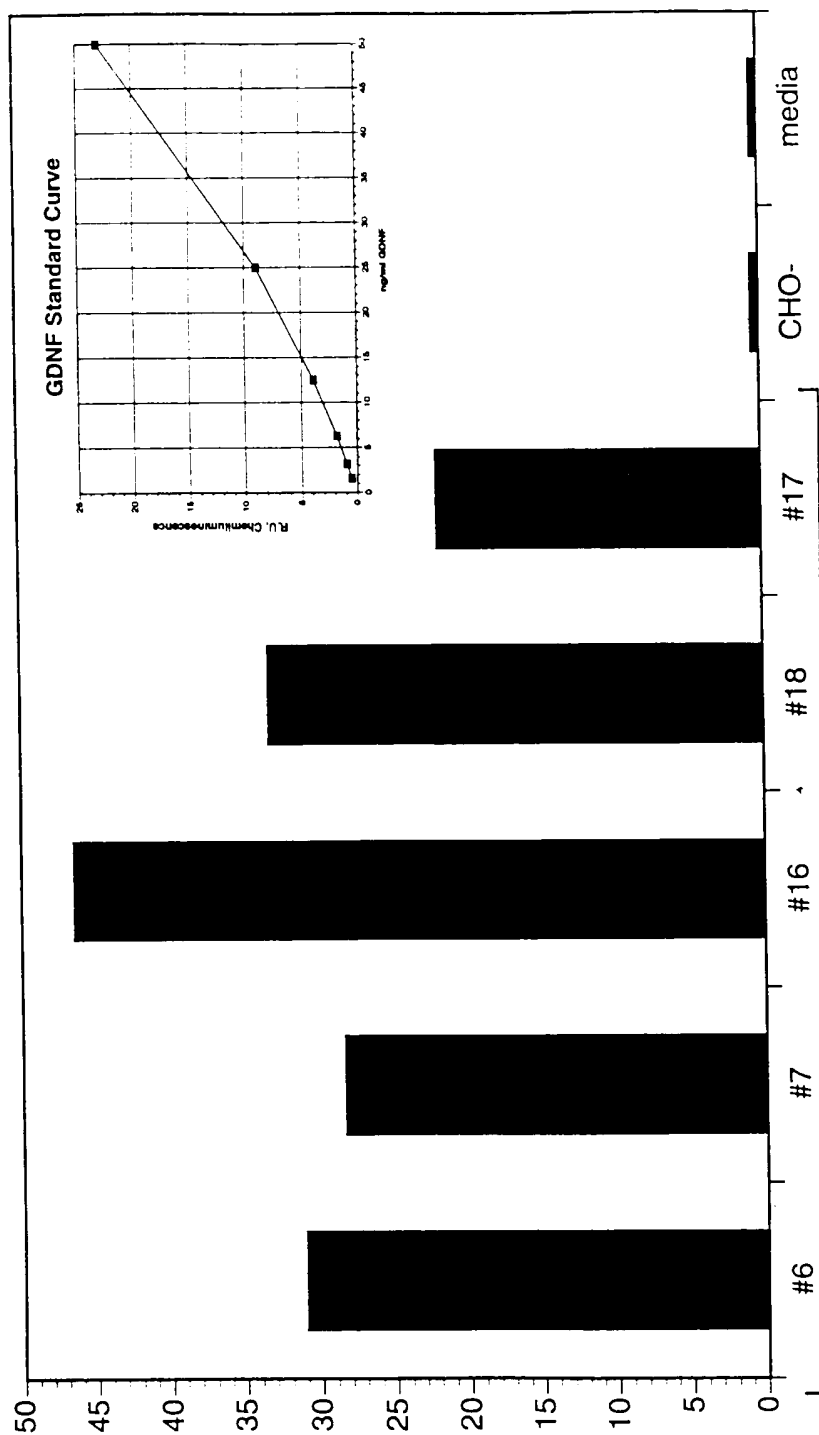


Fig. 9

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Estimated Neublastin Concentration [ng/ml]



CHO Neblastin Clones

Fig. 10

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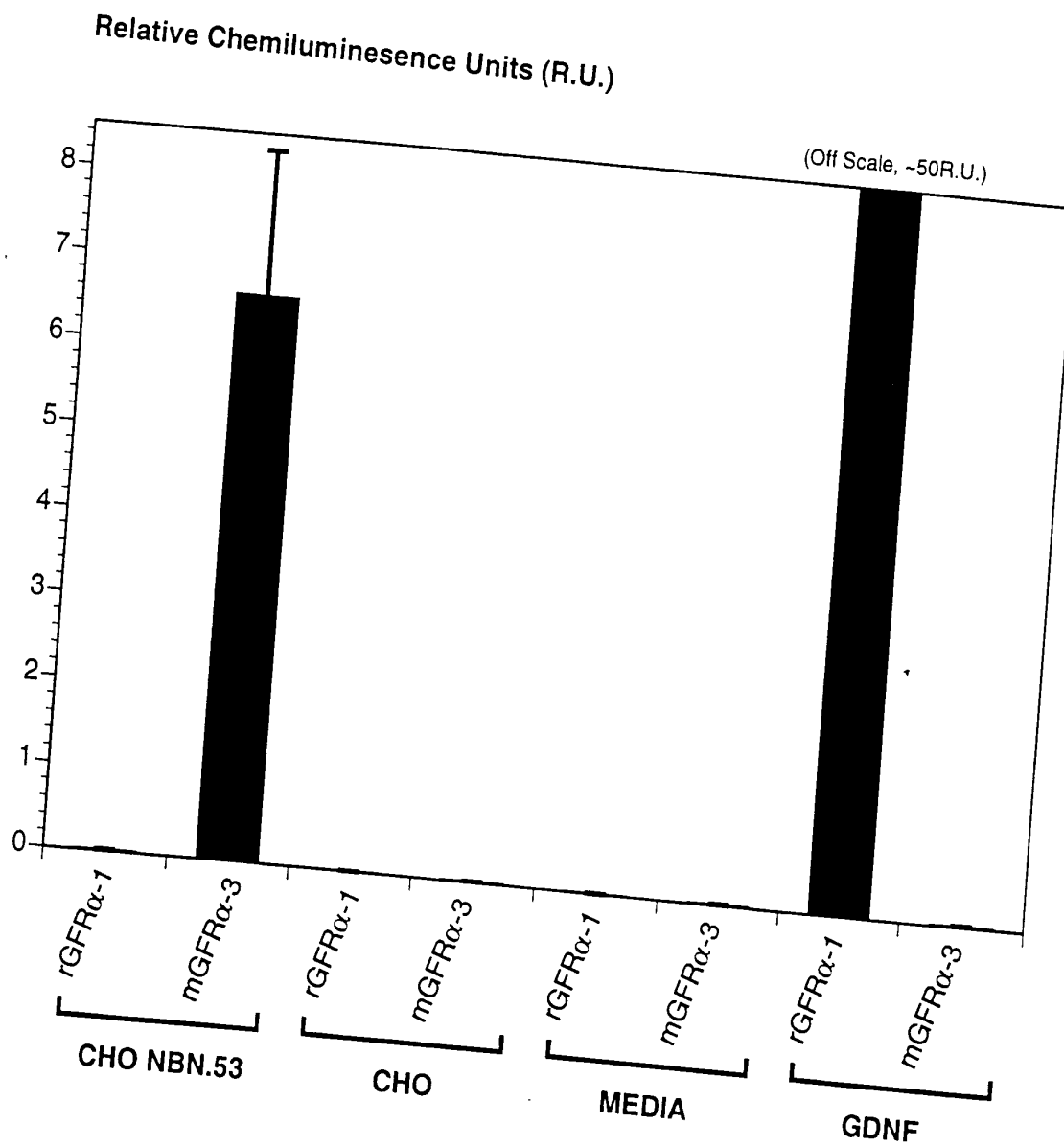
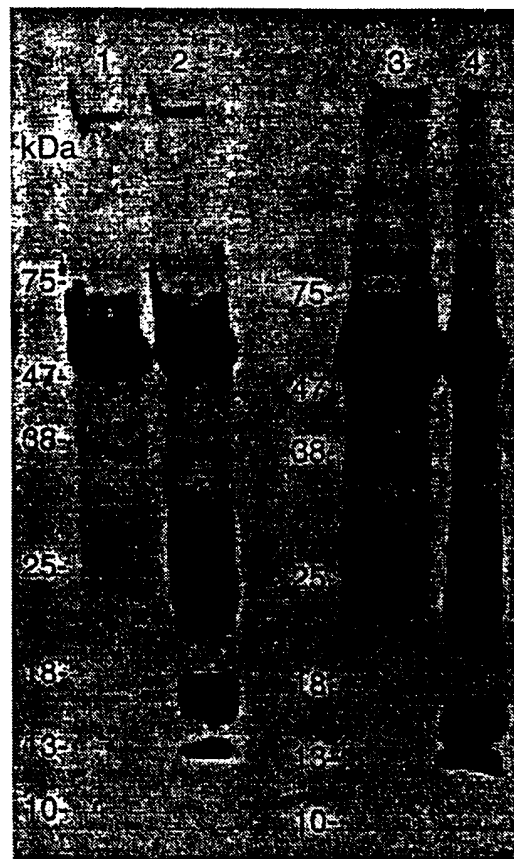


Fig. 11

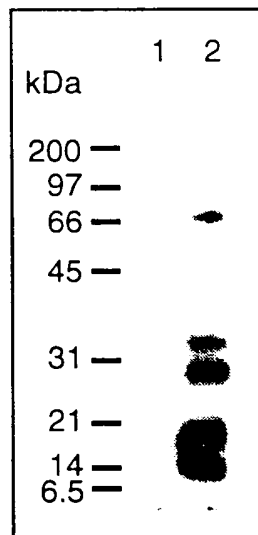
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1. Control medium stained with R30 anti-peptide antibody
2. Neublastin containing conditioned medium stained with R30 anti-peptide antibody
3. Control medium stained with R31 anti-peptide antibody
4. Neublastin containing conditioned medium stained with R31 anti-peptide antibody

Fig. 12

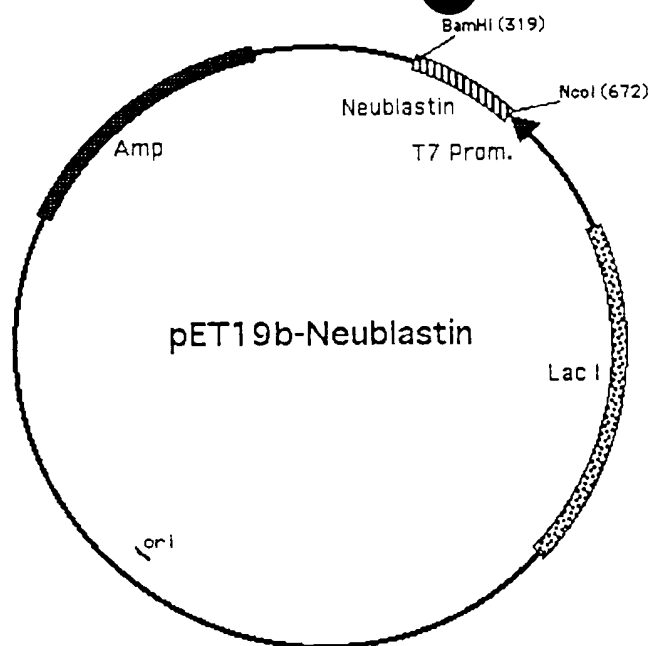
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Extraction of neublastin by affinity-binding on RETL3-Ig
Lane 1: bound from CHO control conditioned media
Lane 2: bound from neublastin overexpressing CHO conditioned media

Fig. 13

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Neublastin Syngene

NcoI (318)

316 TACCATGGCT GGAGGACCGG GATCTCGTGC TCGTGCAGCA GGAGCACGTG GCTGTCGTCT
 ATCTTACCGA CCTCCTGGCC CTAGAGCACG AGCACGTCGT CCTCGTGCAC CGACAGCAGA
 1 ▶ M A G G P G S R A R A A G A R G C R L

376 GCGTTCTCAA CTAGTGCCGG TCGTGCACT CGGACTGGGA CACCGTTCCG ACGAACTAGT
 CGCAAGAGTT GATCACGGCC ACGCACGTGA GCCTGACCCT GTGGCAAGGC TGCTTGATCA
 19 ▶ R S Q L V P V R A L G L G H R S D E L V

436 ACGTTTTTCGT TTTTGTTTCAG GATCTTGTCG TCGTGACGT TCTCCGCATG ATCTATCTCT
 TGCAAAAGCA AAAACAAGTC CTAGAACAGC AGCACGTGCA AGAGGCGTAC TAGATAGAGA
 39 ▶ R F R F C S G S C R R A R S P H D L S L

496 AGCATCTCTA CTAGGAGCCG GAGCACTAAG ACCGCCGCCG GGATCTAGAC CTGTATCTCA
 TCGTAGAGAT GATCCTCGGC CTCGTGATTC TGGCGGCGGC CCTAGATCTG GACATAGAGT
 59 ▶ A S L L G A G A L R P P P G S R P V S Q

556 ACCTTGTTGT AGACCTACTA GATACGAAGC AGTATCTTTC ATGGACGTAA ACTCTACATG
 TGGAACAACA TCTGGATGAT CTATGCTTCG TCATAGAAAG TACCTGCATT TGAGATGTAC
 79 ▶ P C C R P T R Y E A V S F M D V N S T W

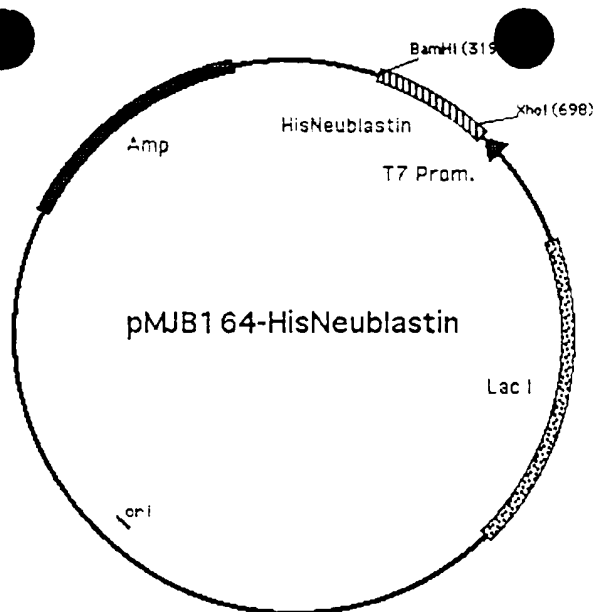
BamHI (671)

616 GAGAACCGTA GATAGACTAT CTGCAACCGC ATGTGGCTGT CTAGGATGAT AATAGGGATC
 CTCTTGGCAT CTATCTGATA GACGTTGGCG TACACCGACA GATCCTACTA TTATCCCTAG
 99 ▶ R T V D R L S A T A C G C L G . . .

676 CGGCT
 GCCGA

Fig. 14

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HisNeubl原因

XhoI (340)

```

301  TACCATGGGC CATCATCATC ATCATCATCA TCATCATCAC TCGAGCGGCC ATATCGACGA
    ATCTTACCCG GTAGTAGTAG TAGTAGTAGT AGTAGTAGTG AGCTCGCCGG TATAGCTGCT
    1► M G H H H H H H H H H S S G H I D D

361  CGACGACAAG GCTGGAGGAC CGGGATCTCG TGCTCGTGCA GCAGGAGCAC GTGGCTGTCTG
    GCTGCTGTTT CGACCTCCTG GCCCTAGAGC ACGAGCACGT CGTCCTCGTG CACCGAÇAGC
    19► D D K A G G P G S R A R A A G A R G C R

421  TCTGCGTTCT CAACTAGTGC CGGTGCGTGC ACTCGGACTG GGACACCGTT CCGACGAACT
    AGACGCAAGA GTTGATCACG GCCACGCACG TGAGCCTGAC CCTGTGGCAA GGCTGCTTGA
    39► L R S Q L V P V R A L G L G H R S D E L

481  AGTACGTTTT CGTTTTTGTT CAGGATCTTG TCGTCGTGCA CGTTCTCCGC ATGATCTATC
    TCATGCAAAA GCAAAAACAA GTCCTAGAAC AGCAGCACGT GCAAGAGGCG TACTAGATAG
    59► V R F R F C S G S C R R A R S P H D L S

541  TCTAGCATCT CTA TAGGAG CCGGAGCACT AAGACCGCCG CCGGGATCTA GACCTGTATC
    AGATCGTAGA GATGATCCTC GGCTCGTGA TTCTGGCGGC GGCCCTAGAT CTGGACATAG
    79► L A S L L G A G A L R P P P G S R P V S

601  TCAACCTTGT TG TAGACCTA C TAGATACGA AGCAGTATCT TTCATGGACG TAAACTCTAC
    AGTTGGAACA ACATCTGGAT GATCTATGCT TCGTCATAGA AAGTACCTGC ATTTGAGATG
    99► Q P C C R P T R Y E A V S F M D V N S T

661  ATGGAGAACC GTAGATAGAC TATCTGCAAC CGCATGTGGC TGTCTAGGAT GATAATAGGG
    TACCTCTTGG CATCTATCTG ATAGACGTTG GCGTACACCG ACAGATCCTA CTATTATCCC
    119► W R T V D R L S A T A C G C L G . .

721  ATCCGGCTGC TAACAAAGCC CG
    TAGGCCGACG ATTGTTTCGG GC
  
```

BamHI (719)

Fig. 15

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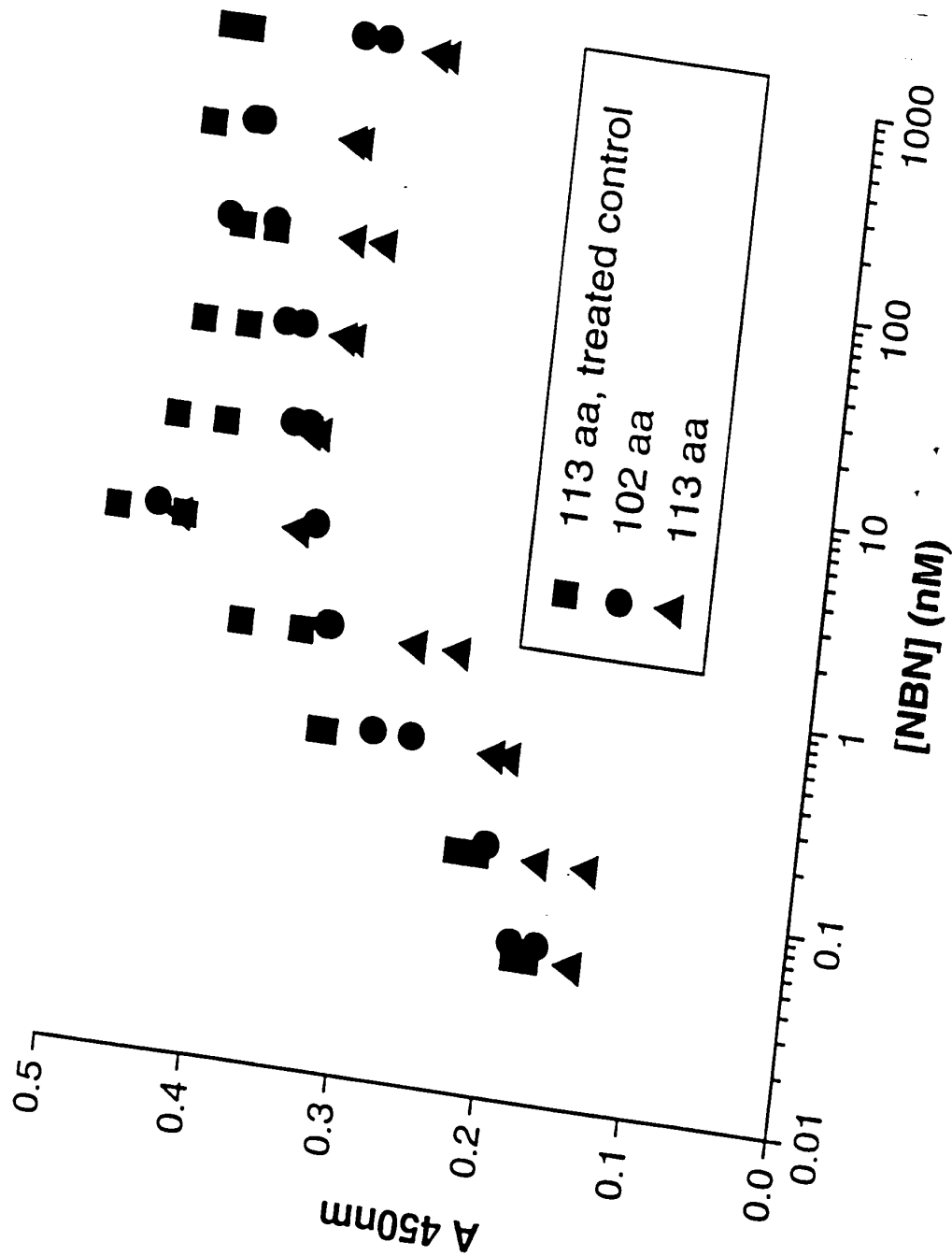
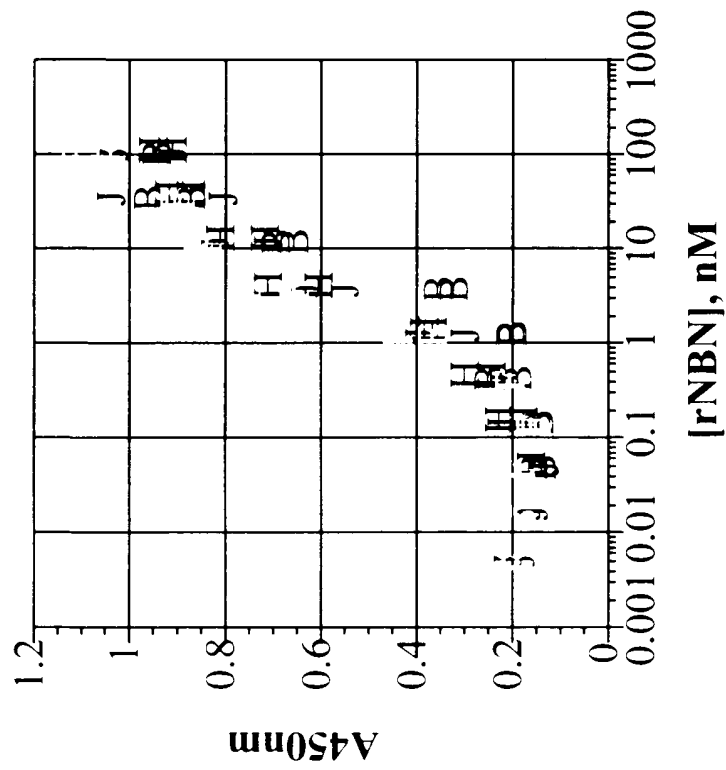


FIG 16

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B R14K (N) 0.15 mg/ml
 J R14K (N-14) 0.22 mg/ml LysC
 H R14K (N-7) 0.88 mg/ml
 WT 1.0 mg/ml (11-16-00 #4)

FIG. 17